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Original communication

Improvement of short tandem repeat analysis of samples highly contaminated by humic acid

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ABSTRACT

We investigated several methods for obtaining successful short tandem repeat (STR) results from high-humic acid (HA)-content samples. DNA purification efficiency was tested for QIAquick® PCR Purification, QIAamp® DNA Investigator and Prepfiler™ Forensic DNA Extraction kits. HA-removal capacity of Inhibitor Remover and InhibitEX® Tablet was tested. Experiments on overcoming HA effects on STR amplification were conducted using an AmpliTaq Gold® DNA Polymerase and a TaKaRa Ex Taq™ Hot Start Version (Ex Taq HS) with BSA addition. QIAquick kit was most efficient in HA removal and Ex Taq HS showed high resistance to HA. Increasing the amounts of Taq polymerases and BSA addition were shown to be efficient in overcoming PCR inhibition, but BSA addition was superior to the former method. Inhibitor Remover and InhibitEX® Tablet did not positively affect the STR results. This study will help achieve better STR results with high-HA-content samples.

1. Introduction

Short tandem repeat (STR) typing can be performed on bones to provide genetic information on personal identification, family relationships and gene flow between populations.[1–3] For successful STR amplification, it is essential to obtain clean DNA without PCR inhibitors. Humic acid (HA), which is one of the primary PCR inhibitors originating from soil, is often co-extracted with DNA after several trials to remove it, especially in old bones. HA can cause false-negative PCR results and incorrect DNA quantification results.[4,5]

HA consists of a mixture of plant and animal residues resulting from chemical and/or biological decomposition.[6,7] Characteristic features of HA are their structural heterogeneity, their ability to bind metal ions by complex formation and their property to interact with a variety of organic compounds.[6] Due to these properties, DNA extracted from samples exposed to the environment can be contaminated by HA. Since HA can inhibit the enzymatic activity of the DNA polymerase and bind the template DNA preventing it from being amplified, PCR inhibition can occur.[8–10]

Various HA-removal methods, such as treatment with aluminum ammonium sulfate, polyvinylpolypyrrolidone and hexadecyltrimethylammonium bromide, have been used.[11] Recently, commercially available DNA purification kits have been widely applied.[12–15] However, PCR inhibitors, including HA, cannot be sufficiently eliminated by using DNA purification kits. For examples, several DNA samples extracted from bones that were about 60 years old using Qiagen Genomic-tips (Qiagen, Hilden, Germany) showed a brownish color.[13] In addition, PCR inhibition was observed in some DNA samples in DNA purification using a

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CleanMix kit (Talent, France).[16] These results demonstrated that it was important to select purification kits that are able to efficiently remove HA for successful STR amplification.

When new DNA purification kits are introduced, manufacturers show the PCR removal efficiency compared to that of other kits using PCR inhibitors, including HA. However, it is still difficult to determine which kit is the best for removal of HA because they used a limited range of PCR inhibitor concentrations for the tests. For example, approximately 2.5 μg of HA was used for stability tests with the Prepfiler™ Forensic DNA Extraction kit (Applied Biosystems, Warrington, UK), DNA IQ™ System (Promega, Madison, WI, USA), EZ1 DNA Investigator kit (Qiagen) and QIAamp DNA Micro kit (Qiagen).[12] Similar results were obtained for the four different kits. It was reported that approximately 0.7–3.3 μg/μL of HA can be recovered in DNA extracted from soils depending on the type of soil and this represents 0.21–0.99% of total HA in the soil.[8,17] Although HA-amounts extracted from soils could not directly correspond to those extracted from forensic samples buried in soils, we speculate from the report that higher amounts of HA could be extracted from samples exposed to soils.

In the present study, we attempted to identify a DNA purification kit that can be used in samples highly contaminated by HA. To achieve this goal, we tested the HA-removal capacities of three DNA purification kits, including the QIAquick® PCR Purification kit (Qiagen), the QIAamp® DNA Investigator kit (Qiagen), and the Prepfiler™ Forensic DNA Extraction kit (Applied Biosystems) at a range of HA concentrations. In addition, we tested the HA-removal properties of two products designed to remove PCR inhibitors: Inhibitor Remover included in the I-genomic Soil DNA Extraction Mini kit (Intron Biotechnology, Korea) and InhibitEX® Tablet included in the QIAamp DNA Stool Mini kit (Qiagen). The Inhibitor Remover is introduced as an effective material to remove PCR inhibitors including higher concentrations of HA in DNA extraction from soil.[18] The InhibitEX Tablet is introduced as a material to absorb DNA-damaging substances and PCR inhibitors present in stool samples.[19] Although this is not its primary purpose, the Stool Mini kit has been used for DNA extraction from bone and teeth.[20] We hypothesize that the InhibitEX Tablet may not be limited to PCR inhibitors observed from stool and attempted to use it to remove HA.

To improve the PCR efficiency of samples containing inhibitors, PCR component modifications can be used. Such methods entail increasing the amount of polymerases, using PCR additives, and using polymerases that are less susceptible to PCR inhibitors.[4,21,22] TaKaRa Ex Taq™ Hot Start Version (Ex Taq HS; Takara, Shiga, Japan) has been shown to be more resistant to HA.[23] Bovine serum albumin (BSA) has also been shown to mitigate PCR inhibition by HA.[7,24] Since the type and amount of polymerase and use of additives, such as BSA, have been shown to be helpful in overcoming the inhibitory effect of HA, we compared two popular polymerases, AmpliTaq Gold and Ex Taq HS together with BSA. This study on HA-removal methods and overcoming the inhibitory effects of HA on PCR will be helpful to obtain better STR results from samples containing a high HA content.

2. Materials and methods

2.1. Sample preparation

K562 DNA (Promega, Madison, WI, USA) and HA (Fluka, Buchs, Switzerland) were prepared. DNA samples were diluted using $T_{10}E_{0.1}$ buffer (pH 8.0). HA was dissolved at 2000 ng/μl in 10 mM NaOH, and the resultant HA solution was further diluted in distilled water. Finally, 0.25 ng/μl DNA samples containing various HA concentrations ranging from 10 to 1000 ng/μl were prepared.

2.2. HA removal using three different DNA purification kits

The QIAquick, Investigator and Prepfiler kits were used to remove the HA from 100 μl DNA samples containing 10–60 μg of HA. All of the methods produced the same final DNA-extract volume, 50 μl. We followed a standard protocol for the QIAquick kit[25] and the cleanup of DNA protocol described in the Appendix for the Investigator kit.[26] For both, we included an additional wash step. For the Prepfiler kit, we added 200 μl of Prepfiler Lysis Buffer to 100 μl of DNA samples, and then followed the Repurification protocol.[27] In this later case, we skipped the additional washing step because the standard protocol contained a three-times washing step. The HA-removal capacities of the three DNA purification kits was compared in terms of the number of amplified STR loci using the AmpFlSTR® Identifiler® PCR Amplification kit (Applied Biosystems). STR amplification was performed in a 10 μl reaction volume containing 4 μl of PCR Reaction Mix, 2.0 μl of Primer Set, 1 U of AmpliTaq Gold and 3.8 μl of DNA extract, according to the manufacturer's PCR conditions.[28] The Identifiler reactions were conducted in duplicate in all of the experiments. The peak height threshold for interpretation of the STR result was 50 RFU.

2.3. Application of Inhibitor Remover and InhibitEX Tablet

Inhibitor Remover and InhibitEX Tablet were applied to DNA samples in a volume of 100 μl containing 40 μg of HA. For the experiments with the Inhibitor Remover, we added 2 μl of the material to DNA samples in a centrifuge tube. The mixture was vortexed and incubated for 5 min, and then centrifuged for 1 min at 13,000 rpm, after which the supernatant was transferred into a new centrifuge tube. For the experiments with the InhibitEX Tablet, we ground the tablet finely in a mortar. Then, 0.1 g of the InhibitEX Tablet and 200 μl of distilled water were added to the DNA samples in a centrifuge tube. The tube was vortexed and incubated for 1 min and then centrifuged for 6 min at 14,000 rpm. Subsequently, about 130–140 μl of supernatant was transferred into a new tube.

DNA was isolated from each supernatant obtained from the Inhibitor Remover and InhibitEX Tablet using the three DNA purification kits. Each purification step was the same as described above. The HA-removal efficacies of Inhibitor Remover and InhibitEX Tablet were compared based on the numbers of amplified STR loci using the Identifiler kit and DNA concentration and C_T value of IPC obtained from a Quantifiler Human DNA Quantification kit (Applied Biosystems). For Quantifiler reactions, we followed the manufacturer's instructions.[29]

2.4. Modification of PCR components

DNA samples containing from 38 to 190 ng of HA were used for the Identifiler reactions. The final HA concentrations ranged from about 3.8 to 19 ng/μl. AmpliTaq Gold and Ex Taq HS were used for amplification, and their amounts increased from 1 to 4 U. Furthermore, about 400 ng/μl of BSA (the final concentration in the Identifiler reactions) (Roche, Mannheim, Germany) was added to the STR amplification reactions.

3. Results and discussion

3.1. HA-removal efficiency of three DNA isolation kits

When HA is not sufficiently removed during the process of DNA purification, a DNA solution that is brownish in color is extracted. Some HA concentration levels can easily be discerned with the unaided eye (Supplementary Data 1). When DNA samples containing 10 μg of HA were tested, the overall DNA solutions extracted

Table 1
Identifiler reaction results using DNA samples obtained from different DNA purification kits.

HA amount (μg)[a]	Number of STR amplified loci		
	QIAquick® PCR Purification kit	Prepfiler™ Forensic DNA Extraction kit	QIAamp® DNA Investigator kit
0	16/16[b]	16/16	16/16
10	16/16	12/11	0/0
20	16/16	0/0	0/0
30	15/14	0/0	0/0
40	11/8	0/0	0/0
50	3/2	0/0	0/0
60	0/0	0/0	0/0

[a] Amount of HA in 100 μl of 250 pg/μl DNA solutions.
[b] The number of amplified loci (1st STR result/2nd STR result) in duplicate amplification.

using the Prepfiler and Investigator kits showed stronger brownish colors than in the case of the QIAquick kit. The three kits showed different STR results (Table 1). All of the 16 STR loci were amplified in duplicate by the QIAquick kit, 11-12 by the Prepfiler kit, and no amplification was achieved using the Investigator kit. The QIAquick kit produced relatively reliable STR results at 30 μg of HA, showing 14–15 amplified STR loci. Excluding no HA and extremely high HA amounts (e.g., 60 μg), significant differences in the number of amplified alleles were detected between the QIAquick kit and the Prepfiler kit (p-value $= 7.8 \times 10^{-4}$ with t-test), as well as between the QIAquick kit and the Investigator kit (p-value $= 8.8 \times 10^{-5}$ with t-test). These results suggest that the QIAquick kit is more efficient than the Prepfiler and Investigator kits in purifying DNA.

The Prepfiler and Investigator kits are designed for forensic case work. They are optimized for the collection of small amounts of DNA and removal of PCR inhibitor amounts that may be typically encountered in that field. The QIAquick kit, by contrast, is designed for purification of PCR products and DNA cleanup. The differing HA-removal capacities of each can be attributed to the distinctive characteristics of each.

Lee et al.[14] reported that when the QIAquick kit and QIAamp® DNA Mini kit were compared, the buffers of the former were more efficient in removal of higher concentrations of HA than those of the QIAamp Mini kit. The AW1 and AW2 buffers are washing buffers included in the QIAamp Mini and Investigator kits. The PE buffer is washing buffer included in the QIAquick kit. These indicate that the PE buffer could have higher ability to remove HA than the AW1 and AW2 buffers. It was difficult to explain why they generate different efficiencies from commercially available kits. However, considering that the PE buffer is included in kits optimized for purification (e.g., QIAquick PCR purification kit, QIAquick Nucleotide removal kit, MinElute PCR Purification kit and QIAquick Gel extraction kit) whereas the AW1 and AW2 buffers are included in DNA isolation kits (e.g., QIAamp DNA Investigator kit, QIAamp DNA Mini/Micro kit and QIAamp DNA Blood Midi/Maxi kit), the PE buffer might be a stronger washing buffer than the AW1 and AW2 buffers.

Although the QIAquick kit showed the highest HA-removal capacity, PCR inhibition was observed in DNA samples containing 30 μg of HA. When DNA samples contained ≥40 μg of HA, PCR was inhibited relatively severely, showing 8–11 amplified STR loci. As these results illustrate, the HA-removal efficacy of any DNA purification kit is inherently limited. Therefore, we applied Inhibitor Remover and InhibitEX Tablet to DNA samples containing ≥40 μg of HA.

3.2. Efficiency of Inhibitor Remover and InhibitEX Tablet

When Inhibitor Remover was applied to DNA samples containing 40 μg of HA, the deep brown color of the DNA-containing solution was completely removed (Supplementary Data 2). The IPC C_T values ranged from 27 to 28 for all of the samples (Table 2). However, Inhibitor Remover negatively affected DNA recovery. When Inhibitor Remover was combined with the QIAquick kit or the Investigator kit, the DNA concentrations could not be determined in all cases. When the Prepfiler kit was used, the DNA concentrations were estimated to be 0.015 ng/μl and 0.002 ng/μl/ND (Not Determined) in the samples containing 0 and 40 μg of HA, respectively. These results also indicated that the method reduced DNA recovery.

Inhibitor Remover is utilized for removal of high HA contents.[18] In our experiments, 2 μl of Inhibitor Remover was used, which was the minimum volume for a clean DNA solution with samples containing 40 μg of HA. Despite adjustments made to the volume of the material, we could not prevent DNA loss. This indicates that the Inhibitor Remover removes not only HA but also certain amounts of DNA. This is significant, because bone samples can contain minute amounts of DNA. Taken together, these results indicated that application of Inhibitor Remover to samples containing minute amounts of DNA is inappropriate, despite its high HA-removal capacity.

When DNA samples containing 40 μg of HA were treated with the InhibitEX Tablet and subsequently applied to the three DNA purification kits, the mean C_T values of IPC were 28.0, Not Determined (ND) and 30.8 for the QIAquick, Prepfiler and Investigator kits, respectively (Table 2). The numbers of amplified STR loci were

Table 2
Quantifiler and Identifiler reaction results after the application of Inhibitor Remover or InhibitEX Tablet.

HA removal materials	Kits tested	HA amount (μg)[a]	DNA recovery (ng/μl)	IPC (C_T)[b]	STR results
Inhibitor Remover	QIAquick® PCR Purification kit	0	ND[c]	28.185 ± 0.007[d]	0/0[e]
		40	ND	28.130 ± 0.127	0/0
	Prepfiler™ Forensic DNA Extraction kit	0	0.015 ± 0.003[d]	28.060 ± 0.028	10/12
		40	0.002/ND	28.000 ± 0.014	0/1
	QIAamp® DNA Investigator kit	0	ND	27.865 ± 0.007	0/0
		40	ND	27.915 ± 0.078	0/0
InhibitEX Tablet	QIAquick® PCR Purification kit	0	0.176 ± 0.017	27.895 ± 0.049	16/16
		40	0.070 ± 0.005	28.015 ± 0.049	16/16
	Prepfiler™ Forensic DNA Extraction kit	0	0.268 ± 0.039	27.895 ± 0.049	16/16
		40	0.002 ± 0.002	ND	0/0
	QIAamp® DNA Investigator kit	0	0.012 ± 0.005	28.280 ± 0.184	7/8
		40	0.014 ± 0.005	30.830 ± 0.495	8/8

[a] Amount of HA in 100 μl of 250 pg/μl DNA solutions.
[b] C_T: the threshold cycle for log phase amplification of IPC.
[c] ND: Not determined.
[d] The means of real-time PCR results in duplicate.
[e] The number of amplified loci (1st STR result/2nd STR result) in duplicate amplification.

16/16, 0/0 and 8/8, respectively. These results showed that the method combining InhibitEX Tablet and the QIAquick kit was most efficient for STR typing. When this combination method was compared with the method using only the QIAquick kit, the STR results of the former were better than the STR results of the latter (Table 1).

We investigated possible reasons for the improved STR results when the combination method was used, and found that the most important factor was not InhibitEX Tablet's high HA-removal capacity but rather the experimental design, which prevented the InhibitEX Tablet from reducing the DNA sample volume.

From the pilot test results, we confirmed that the InhibitEX Tablet reduces the total volume of DNA samples. To minimize this effect, we modified the manufacturer's protocol.[19] According to the protocol of the Stool Mini kit, approximately 0.5 g of the InhibitEX Tablet is added to 1.4 ml of lysis solution containing stool. In our study, however, we added 0.1 g of the InhibitEX Tablet and 200 μl of distilled water to 100 μl of DNA sample. After the InhibitEX Tablet reaction was completed, 130–140 μl of the DNA samples were obtained, and DNA was extracted from those samples using the three DNA purification kits.

In these experiments, the InhibitEX did not absorb HA separately but absorbed the total DNA sample volume containing HA; accordingly, DNA samples containing small amounts of HA were used for DNA extraction, which allowed for better STR results. Indeed, this mechanism was confirmed by additional experiments. We took 140 μl of the same 300 μl of DNA sample that was used for the InhibitEX Tablet/QIAquick kit combination method and applied it to the QIAquick kit without the InhibitEX Tablet. Quantifiler results showed that the mean DNA concentration was 0.096 ± 0 ng/μl, and the mean C_T value of IPC, 30.095 ± 0.573. The number of amplified STR loci was 16/16. When these results were compared with those obtained using the InhibitEX Tablet/QIAquick kit combination method, the STR results were the same. Also, no considerable differences in the DNA recovery yield values or C_T values of IPC were observed (Table 2).

The InhibitEX Tablet is an optimized material for PCR inhibitors, such as bile salt and complex polysaccharides observed from stool.[30] Due to this property, the InhibitEX Tablet might be not compatible with HA. Lee et al.[20] reported that DNA was obtained successfully using the Stool Mini kit for DNA extraction from bone and teeth samples. The specific effectiveness of the InhibitEX Tablet to remove HA is not known because they used the whole Stool Mini kit and applied it to relatively fresh samples who were buried in the past 1–6 years. However, it might be possible that the InhibitEX Tablet can remove other PCR inhibitors observed from bone samples. We need further study about the issues.

3.3. STR results with modification of PCR components

Modifications of the STR amplification components showed different STR results (Table 3 and Fig. 1). At 19 ng/μl of HA, 11–12 loci were amplified by 4 U of AmpliTaq Gold and 16 loci were amplified by 2 U of Ex Taq HS. Significant differences in the number of amplified alleles were detected in tests using AmpliTaq Gold or Ex Taq HS at relatively high concentrations of HA (e.g., 11.4 ng/μl, 15.2 ng/μl, and 19.0 ng/μl), (p-value $= 5.8 \times 10^{-4}$ with t-test). These results showed that the Ex Taq HS was more resistant to HA than AmpliTaq Gold, the most common polymerase in commercial STR typing kits. These findings were similar to those of an earlier study that reported Ex Taq HS to be resistant to higher concentrations of skeletal inhibitors.[23] According to the AmpliTaq Gold and Ex Taq HS manuals, both are hot-start Taq polymerases. The compositions of the storage buffers in the two polymerases, that is, Tris–HCl, KCl, EDTA, DTT, Tween 20 and glycerol, are the same, except for Nonidet P-40, which is included only in Ex Taq HS.[31,32] Nonidet P-40 is a PCR additive and has been used in PCR amplification of DNA extracted from soil.[33,34] We believe that Nonidet P-40 may contribute to the difference in overcoming the effects of HA between the two DNA polymerases.

Increases in the amounts of both Taq polymerases improved the STR amplification efficiency. At 15.2 ng/μl of HA, all of the STR loci were amplified by increasing the AmpliTaq Gold units from 1 U to 3 U and by increasing the Ex Taq HS units from 1 U to 2 U. However, there were some limitations to overcoming PCR inhibition by increasing the AmpliTaq Gold units to 4 U, which resulted in 11–12 amplified loci at 19 ng/μl of HA.

In tests with BSA addition at 15.2 ng/μl of HA, 16 full STR loci were amplified by 1 U of AmpliTaq Gold with BSA, whereas without BSA, only 1 STR locus was amplified. At 19.0 ng/μl of HA, 16 STR loci were amplified when using 2 U of AmpliTaq Gold with BSA were used, whereas only 3–4 loci were amplified without BSA. The similar results were obtained by using 1 U of Ex Taq HS with BSA. Significant differences (p-value $= 5.8 \times 10^{-3}$ with t-test) in the number of amplified alleles with or without BSA were detected for extremely high concentration of HA (e.g., 19.0 ng/μl), even with different polymerases. These results showed that the addition of BSA increased the number of detected alleles for higher concentrations of HA. Several of the substances whose inhibition is relieved by BSA in the PCR contain phenolic groups.[7] Phenols are known to bind to proteins by forming hydrogen bonds with peptide bond oxygens.[35] HA is a mixture of polyphenolic substances produced during the degradation of organic matter.[6,7] Therefore, BSA may be able to bind HA and thereby prevent HA binding and inactivation of Taq DNA polymerase.[7]

When the methods of BSA addition were compared with increasing the amounts of Taq polymerases in the aspect of the

Table 3
Identifiler reaction results using DNA samples including various HA concentrations.

HA (ng/μl)[a]	AmpliTaq Gold® DNA Polymerase						TaKaRa Ex Taq™ Hot Start Version			
	1 U		2 U		3 U	4 U	1 U		2 U	
	Normal	+BSA[b]	Normal	+BSA	Normal	Normal	Normal	+BSA	Normal	+BSA
0	16/16[c]	16/16	16/16	16/16	16/16	16/16	16/16	16/16	16/16	16/16
3.8	16/16	16/16	16/16	16/16	16/16	16/16	16/16	16/16	16/16	16/16
7.6	15/15	16/16	16/16	16/16	16/16	16/16	16/16	16/16	16/16	16/16
11.4	5/6	16/16	16/16	16/16	16/16	16/16	16/16	16/16	16/16	16/16
15.2	1/1	16/16	8/11	16/16	16/16	16/16	13/13	16/16	16/16	16/16
19.0	0/0	14/13	4/3	16/16	10/8	11/12	7/7	16/16	16/16	16/16

[a] The value is the final concentration of HA in the STR amplification reaction. The final HA concentrations ranging from 3.8 to 19 ng/μl corresponded to HA amounts ranging from 38 to 190 ng in 10 μl of the Identifiler reactions.
[b] BSA: addition of BSA to Identifiler reaction.
[c] The number of amplified loci (1st STR result/2nd STR result) in duplicate amplification.

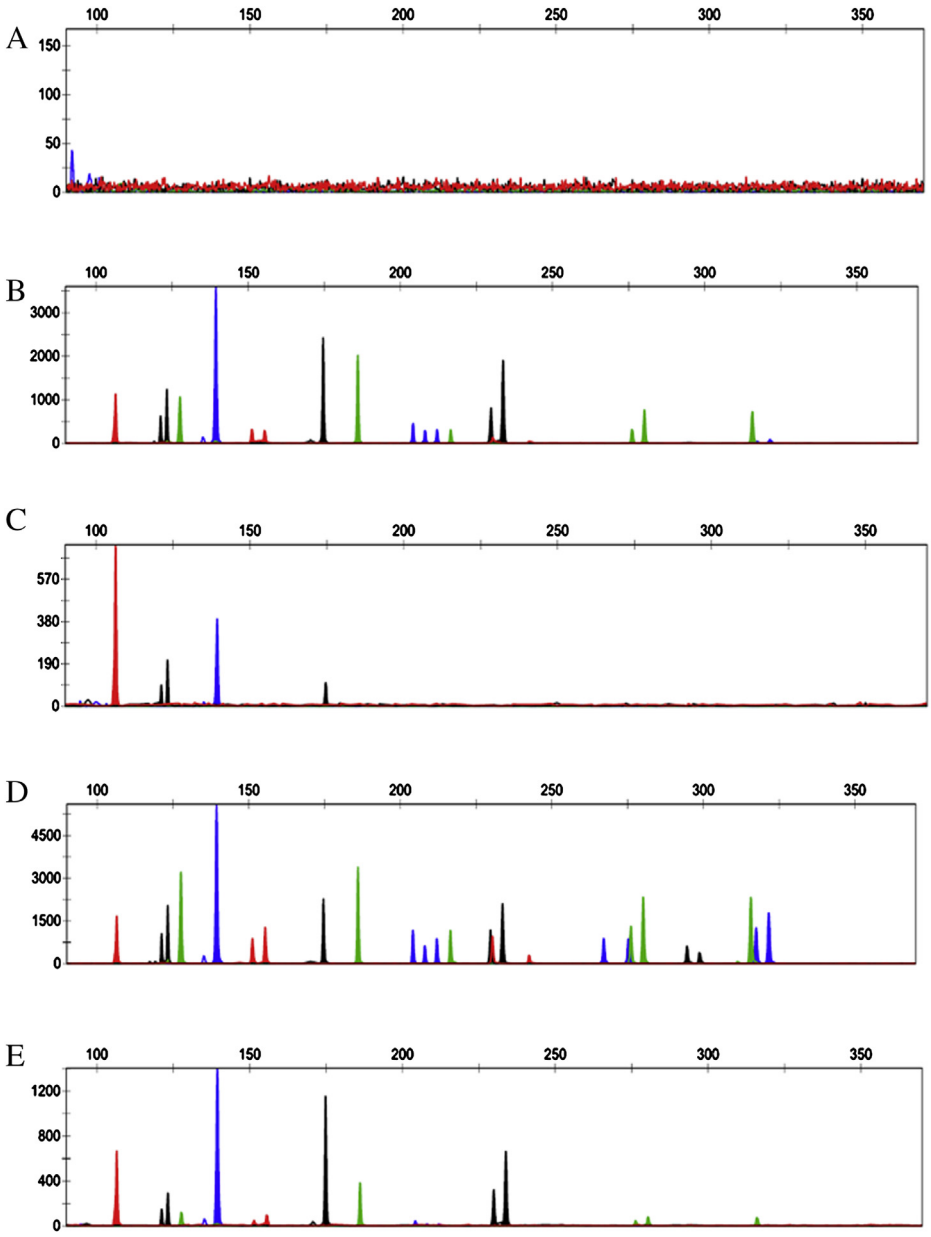

Fig. 1. Representative electropherograms of STR results obtained under different amplification conditions. Panels A to J show the results of the Identifiler kit reactions at an HA concentration of 19.0 ng/μl (final concentration in STR reaction). The reactions were carried out using 1 U of AmpliTaq Gold (A), 1 U of AmpliTaq Gold with BSA (B), 2 U of AmpliTaq Gold (C), 2 U of AmpliTaq Gold with BSA (D), 3 U of AmpliTaq Gold (E), 4 U of AmpliTaq Gold (F), 1 U of Ex Taq HS (G), 1 U of Ex Taq HS with BSA (H), 2 U of Ex Taq HS (I), and 2 U of Ex Taq HS with BSA (J).

effectiveness in overcoming HA effects, BSA addition seemed to be more efficient. At 19 ng/μl of HA, the mean numbers of amplified loci increased from 0 to 11.5 when the AmpliTaq Gold units were increased from 1 U to 4 U. On the other hand, at the same HA concentration, 16 loci were amplified using 2 U of AmpliTaq Gold with BSA. These results suggest that for samples containing higher concentrations of HA, adding 400 ng/μl of BSA may be more efficient than the additional use of Taq polymerases.

In regards to using BSA to limit PCR inhibition, one study reported that the PCR yield was increased when using up to 800 ng/μl of BSA.[24] Another study reported that the optimum concentration of BSA for limiting PCR inhibition was 400 ng/μl.[7] It is not known whether the PCR buffer of the Identifiler kit contains BSA, or at what concentrations, because the kit manufacturer does not provide this information. Thus, in a pilot study, we examined the STR amplification efficiency by applying 400 ng/μl and 800 ng/μl of BSA with 1 U of AmpliTaq Gold to DNA samples containing 40 ng/μl of HA. The results of this analysis showed the same 16 STR loci were amplified for both reactions, and there was no considerable difference. Thus, we used 400 ng/μl of BSA for our STR amplification reactions.

It is known that HA inhibits PCR by binding the template DNA preventing it from being amplified and inhibiting the enzymatic activity of the DNA polymerase.[8–10] From our studies, we determined the enzymatic activity of the DNA polymerase in samples containing HA may be different depending on the type and amount of polymerases, and the efficiency of BSA to reduce inhibition. Opel et al.[10] revealed that the level of inhibition of HA is related to amplicon size and primer melting temperature sequence. Therefore, primers designed to produce short amplicons and to have higher

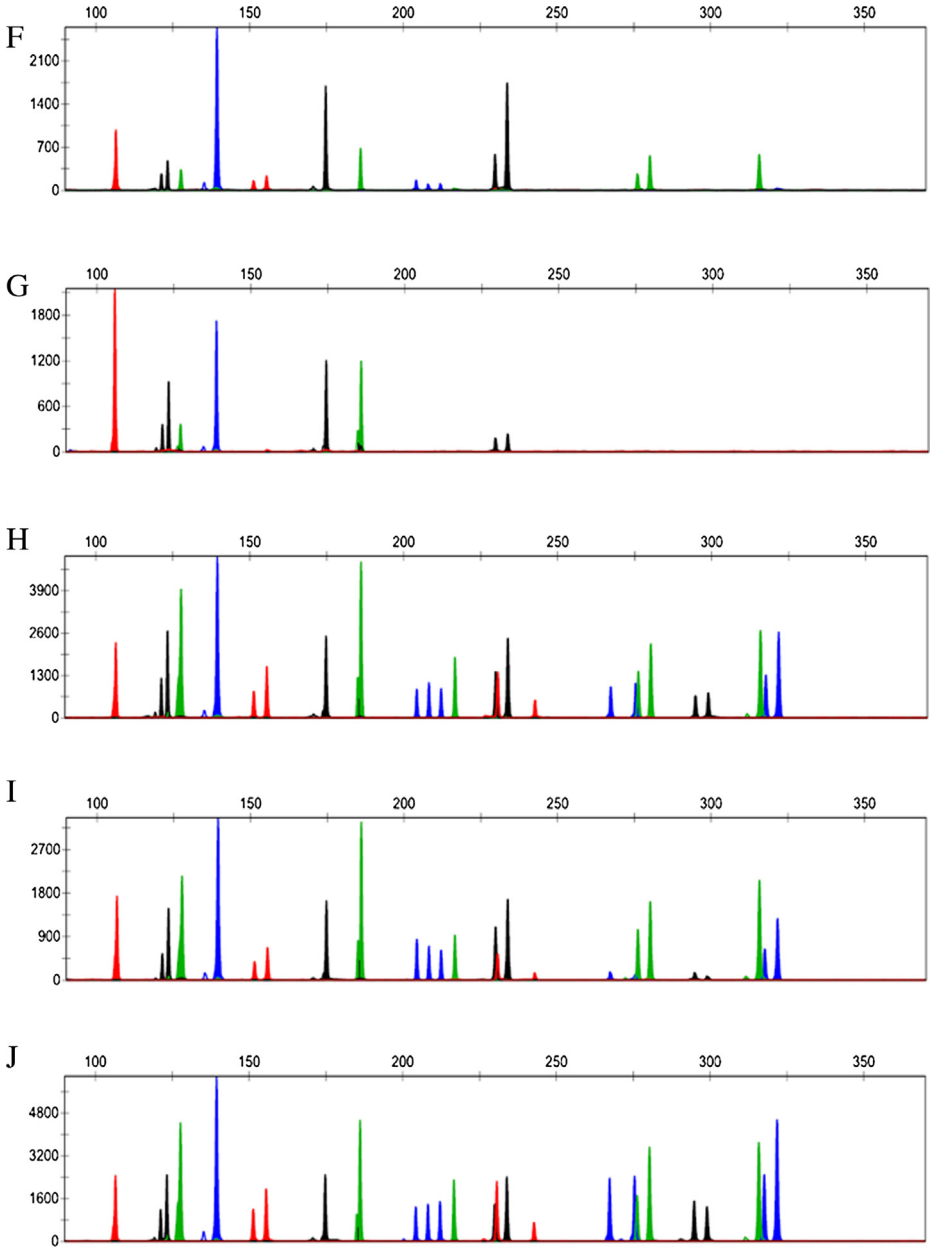

Fig. 1. (*continued*).

melting temperatures,[5,10] with our modification of PCR components, could generate less interference with STR amplification by HA.

For the purposes of overcoming some levels of PCR inhibition, modifications of PCR chemicals is more appropriate than methods involving removal of PCR inhibitors. Whereas modification of PCR chemicals does not affect DNA recovery, PCR removal methods, which involve additional DNA purification steps or application of removers such as Inhibitor Remover or InhibitEX Tablet, can cause DNA loss. This factor is particularly relevant to the achievement of successful STR typing results in cases of old samples, where the DNA contents are limited.

4. Conclusion

Based on the results of this study, the QIAquick kit was determined to be the best DNA purification method for removing HA, and Ex Taq HS was more effective than AmpliTaq Gold in overcoming the effects of HA. Increasing the amounts of Taq polymerases and BSA addition were both found to be efficient in overcoming PCR inhibition, although BSA addition was determined to be superior to the former method. The results obtained from this study will likely prove helpful to those seeking to achieve better STR results from bone samples containing a high HA content.

Ethical approval

None declared.

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Conflict of interest

No conflicts of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jflm.2013.08.001.

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